

PERMANENT GENETIC RESOURCES

Identification of 21 polymorphic microsatellites in the African parasitoid wasp, *Psytalia lounsburyi* (Silvestri) (Hymenoptera: Braconidae)

M. C. BON,* W. JONES,* C. HURARD,* A. LOISEAU,† N. RIS,‡ C. PICKETT,§ A. ESTOUP† and X. FAUVERGUE‡

*USDA-ARS-European Biological Control Laboratory (EBCL), Campus International de Baillarguet CS 90013, 34988 Montferrier le Lez, France, †Centre de Biologie et de Gestion des Populations, INRA, Campus International de Baillarguet CS 30016, 34988

Montferrier le Lez, France, ‡INRA, 400 Route des Chappes, BP 167, 06 903 Sophia Antipolis cedex, France, §California Department of Food and Agriculture (CDFA), Sacramento, CA 95814, USA

Abstract

We have developed 21 dinucleotide repeat microsatellite loci from African populations of *Psytalia lounsburyi* (Silvestri) (Hymenoptera: Braconidae), a parasitoid wasp of the olive fruit fly, as part of a study assessing the role of introgression/hybridization in the success of a biological control introduction. We proposed suitable conditions for polymerase chain reaction multiplexing. All 21 loci were polymorphic with two to 21 alleles per locus within the Kenyan and South African populations tested. Most of them were successfully amplified in two other *Psytalia* species.

Keywords: biological control, hybridization, introgression, olive fruit fly, *Psytalia lounsburyi*

Received 4 November 2007; revision accepted 19 December 2007

Adaptation to new environments is crucial for the success of biological invasions, including the intentional introductions of biological control agents. The multiple step introduction of a biological control agent may lead to reduced fitness and severe bottlenecks in population size (Hufbauer & Roderick 2005). Furthermore, the role of introgression/hybridization among genetically differentiated populations has yet to be demonstrated in the adaptation of a new biological control agent to new environments (Facon *et al.* 2006). *Psytalia lounsburyi* Silvestri (Hymenoptera: Braconidae) is a useful model for assessing this hypothesis. In Africa, it is a koinobiont parasitoid of olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), a primary pest of olives both in the Mediterranean basin and California, USA. It was first identified as a potential biological control agent for this pest as part of a Californian-wide management programme (Daane *et al.* 2008), and will be evaluated in multiple field tests within the Mediterranean basin.

Here, we present the 21 polymorphic microsatellite loci that are optimized for polymerase chain reaction (PCR)

multiplexing in *P. lounsburyi* and were tested for amplification in two other *Psytalia* species.

For construction of the enriched DNA libraries, we followed Kijas *et al.* (1994) with modifications outlined below. Two and a half micrograms of genomic DNA were isolated from seven individuals coming from the ninth generation of a South African population of *P. lounsburyi* maintained at the European Biological Control Laboratory (EBCL). The QIAGEN DNeasy Tissue DNA extraction kit was used before digestion with the *RsaI* restriction enzyme. Only *RsaI* fragments of 300–900 bp were ligated to *Rsa21* and 5′-phosphorylated *Rsa25*, and then pre-amplified by PCR using *Rsa21* as a primer. PCR products were purified with the PCR purification kit QIAGEN and enriched using streptavidin-coated magnetic beads (Promega) and 3′-biotinylated (TC)₁₀ or (TG)₁₀. Enrichments were PCR amplified, priming with *Rsa21*, in order to recover repeating units of DNA fragments. PCR fragments were ligated into a pGEM-T easy vector (Promega) used to transform JM109 high efficiency competent cells (Promega). A total of 960 recombinant clones were picked randomly, and 567 were amplified using a combination of three primers T7, SP6 and (CT)₁₀ or (CA)₁₀. Of these 567 clones, 398 (70%) gave a positive signal. The inserted fragments were sequenced in

Correspondence: Marie-Claude Bon, Fax: +33 (0) 499623049; E-mail: mcbon@ars-ebcl.org

198 clones which showed an appropriate size, that is, 500–1000 bp, of the resulting amplified fragments. Sequencing reactions were performed in the forward and reverse direction using the BigDye version 3.1 dye-terminator system (Applied Biosystems) by Genoscreen and visualized using an ABI 3730 DNA Analyser (Applied Biosystems). All sequenced clones except for three, harboured simple sequence repeats. Sequence redundancy reading showed that 82% of the sequenced clones were uniclones. Primer pairs for 48 loci were designed using OLIGO 4.0 software (National Biosciences Inc). The forward strand primer of

each microsatellite was labelled with one of the three fluorescent dyes (FAM, HEX or NED). Loci were tested on a subset of eight individuals from both Kenyan and South African populations using MegaBACE DNA sequencer (Amersham BioSciences). Individual DNA extractions were performed following a cetyltrimethyl ammonium bromide (CTAB)-based protocol (Doyle & Doyle 1987). Twenty-one polymorphic loci with a low number of aspecific and or stutter bands were retained. GenBank Accession numbers and primer sequences are given for each selected locus in Table 1. Loci were successfully

Table 1 Characteristics of 21 microsatellite loci in females collected in two African populations of *Psytalia lounsburyi*. The table includes locus name, primer pairs, repeat motif, range of allele sizes detected, number of alleles detected (A), number of individuals genotyped (n), observed (H_O) and expected (H_E) heterozygosities, and the significance for the exact test for Hardy Weinberg equilibrium (HWE P value) as implemented in GENEPop version 3.4 (1000 iterations of the Markov chain). Only a P value of < 0.002 for departure from HWE would be considered significant following a Bonferroni correction for multiple comparisons. *, no test performed due to monomorphic locus in the population. Set number refers to one of the three PCR conditions defined for each locus tested separately. Genbank Accession numbers for the original clone sequences are given below each locus name.

Locus name, Genbank Accession no. and (set no.)	Primer sequence (5'–3')	Clone repeat motif and size (bp)	Range of allele sizes (bp)	Kenya A (n)	Kenya H_O H_E	Kenya HWE P value	South Africa A (n)	South Africa H_O H_E	South Africa HWE P value
Pslo1, EU194466 (1)	F: NED-GATGTGAAGAAGGAGGAAGTTG R: GAGTGACGCCTCTAGCATTG	(TC) ₁₂ 4C(TC) ₄ 339	327–349	6 (27)	0.593 0.598	0.419	4 (25)	0.280 0.478	0.0169
Pslo2, EU194467+ (1)	F: NED-GTGAACGTGCTCAGTGGTTG R: TGTCTAGTGTGCCAAGCCAG	(TG) ₁₂ 172	168–198	8 (28)	0.786 0.766	0.232	4 (25)	0.44 0.486	0.3912
Pslo3, EU194468 (1)	F: FAM-TGGAATCGGTGAGATAGGATG R: GTAGAGCTGCGGAGGTGAG	(TC) ₁₆ 176	168–202	12 (28)	0.464 0.641	0.1158	4 (25)	0.480 0.432	1
Pslo4, EU194469 (1)	F: HEX-GTGGTGAAGAGGTGGCTTC R: AGCCTCCTCTTAACGATCTAC	(TC) ₁₂ 112	110–124	5 (28)	0.679 0.671	0.892	3 (25)	0.280 0.251	1
Pslo5, EU194470 (1)	F: FAM-TGACCTATGAGTTACCCCTACG R: GAAGATATTGCTAGCGATGAG	(TC) ₁₄ 320	322–338	6 (27)	0.704 0.655	0.401	3 (25)	0.120 0.117	1
Pslo6, EU194471 (1)	F: NED-TCGGTTTATTCGATGCATTCTC R: CTTACGGTCTTAATCTAGTCAG	(TC) ₃ TG(TC) ₁₂ 260	248–300	11 (27)	0.63 0.70	0.370	8 (25)	0.72 0.804	0.09
Pslo7, EU194472 (1)	F: HEX-TCGCGATGAACCCCACTAAG R: GTTACACGCGGTGTGATTCTAC	(TC) ₂₁ 263	233–283	7 (28)	0.607 0.695	0.173	10 (25)	1.00 0.857	0.685
Pslo8, EU194473 (1)	F: FAM-AGATTTCAGTCATCGGAATGC R: CTAATTCCCATGTTAATGAGTC	(TC) ₁₇ 292	292–324	14 (26)	0.808 0.896	0.342	4 (24)	0.667 0.707	0.613
Pslo9, EU194474 (1)	F: HEX-CACCAAAATGATGAATAAGTCATG R: GTTACATGACAATCTTGAGATG	(TC) ₁₀ 129	123–179	17 (28)	0.714 0.740	0.451	3 (24)	0.083 0.082	1
Pslo10, EU194475 (1)	F: FAM-CATCAAGCCACAGGGTAG R: GTGCTTGCCACGTGAGGATC	(TC) ₁₅ 127	115–151	7 (28)	0.607 0.77	0.02	8 (25)	0.80 0.786	0.268
Pslo11, EU194476 (1)	F: FAM-CTGGATCATGTTAAGGCAGTG R: TGACACGGTGTCTCATGATG	(TC) ₂₀ 169	157–233	21 (28)	0.786 0.96	0.004	11 (25)	0.72 0.807	0.02
Pslo12, EU194477 (3)	F: HEX-TCCTATCAATCTTCAGTTACC R: AGATTACAATCATTTGACGTGC	(TC) ₉ 158	156–165	6 (28)	0.714 0.756	0.01	1 (24)	*	*
Pslo13, EU194478 (1)	F: NED-TCACAAGTTTCCAAGTGCCCTC R: ATCACCATAACCCACCGAAC	(TC) ₂₀ 238	220–268	19 (28)	0.75 0.932	0.06	10 (24)	0.833 0.873	0.04
Pslo14, EU194479 (1)	F: HEX-GAATAGTGGGAGATGAGTGC R: CAACCGTGCGAAGCGATTG	(TC) ₈ 224	222–228	4 (28)	0.536 0.684	0.2	1 (24)	*	*
Pslo15, EU194480 (1)	F: NED-ACAGCTGAACCTTCAACATG R: CTGGGATTCTCTACCGTCAC	(TC) ₁₉ 332	312–362	13 (28)	0.786 0.872	0.32	9 (24)	0.75 0.782	0.681
Pslo16, EU194481 (1)	F: NED-AGGCGACACGCACTCCAATG R: ACTCGACTGAATTAAGTGGTG	(TC) ₁₁ 113	113–161	20 (28)	0.964 0.938	0.546	8 (25)	0.52 0.548	0.455
Pslo17, EU194482 (1)	F: FAM-ATCCAACCTGGTATCAACTCC R: GATACGACACTCCTCATTTGG	(TC) ₁₅ 229	223–285	18 (28)	0.929 0.923	0.764	15 (25)	0.92 0.874	0.659
Pslo18, EU194483 (1)	F: HEX-GTTCTCCAAGTGAATGTCAAC R: TGTTCCTCCGTGAGATTTGC	(TC) ₃ G(CT) ₈ 227	223–233	5 (28)	0.857 0.748	0.228	2 (25)	0.2 0.184	1
Pslo19, EU194484 (2)	F: HEX-CTTTGTGCGTGAAATTGGGATG R: ATGTATGGGACTTCGAAACCTC	(TC) ₇ 8nts(TC) ₈ 295	293–299	3 (27)	0.259 0.338	0.357	2 (25)	0.56 0.509	0.698
Pslo20, EU194485 (2)	F: FAM-TGGAGCCTCACCTGTTGATC R: TGCAGTGCCTGCCAACGGTC	(TC) ₁₁ 302	298–302	5 (27)	0.556 0.628	0.07	2 (25)	0.44 0.497	0.686
Pslo21, EU194486 (3)	F: HEX-TGCCAAGTCTCTCAGCGATC R: ATGGGACACGATAGTAGGATC	(TC) ₁₄ 87	73–91	5 (26)	0.731 0.671	0.553	4 (23)	0.737 0.739	0.209

Table 2 Cross-amplification success of the 21 polymorphic loci cloned in *Psytalia lounsburyi* on two other *Psytalia* species. Successful PCR amplification of a locus in a specimen is represented by a '+', unsuccessful amplification is represented by a '-'

Locus	Pslo 1	Pslo 2	Pslo 3	Pslo 4	Pslo 5	Pslo 6	Pslo 7	Pslo 8	Pslo 9	Pslo 10	Pslo 11	Pslo 12	Pslo 13	Pslo 14	Pslo 15	Pslo 16	Pslo 17	Pslo 18	Pslo 19	Pslo 20	Pslo 21
<i>P. concolor</i>	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+
<i>P. ponerophaga</i>	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+	+	+	-	-	+	-

amplified using a PerkinElmer 9700 and the multilocus amplification kit (QIAGEN) in a 10- μ L volume containing 1 \times QIAGEN Multiplex Master Mix, 0.2 μ M of each primer and 2 μ L of genomic DNA (~10 ng), following Fournier *et al.* (2005). According to the loci considered, three sets of PCRs were defined as follows: initial denaturation at 95 °C for 15 min; 35 cycles of 30 s at 94 °C, 90 s at 60 °C for sets 1 and 2 or 90 s at 57 °C for set 3, 60 s at 72 °C, and 30-min final extension step at 72 °C. Set 1 differed from set 2 by the number of cycles, that is, 30.

Polymorphism at the 21 microsatellite loci was assayed on female individuals of *P. lounsburyi* collected in 2005 from wild populations of Kenya ($n = 28$) and South Africa ($n = 25$). Tests for deviation from Hardy–Weinberg equilibrium and linkage disequilibrium were processed using GENEPOP version 3.4 and Markov chain parameters as follows: dememorization 1000, batches 200, iterations per batch 1000 (Raymond & Rousset 1995). Both tests were corrected for multiple comparisons by a sequential Bonferroni correction (Rice 1989). The conservation of the primer sequences was also examined in two other *Psytalia* species, including an African *P. concolor* (Szépligeti) specimen and a Pakistani *P. ponerophaga* (Silvestri) specimen following the conditions described above.

All loci were polymorphic with three to 21 alleles per locus in the Kenyan population, and the expected heterozygosity ranged from 0.338 to 0.96 (Table 1). No significant linkage disequilibrium among the pairs of loci when pooled across populations was detected after sequential Bonferroni adjustment. Nineteen loci out of 21 were polymorphic with two to 15 alleles per locus in the South African population and the expected heterozygosity ranged from 0.082 to 0.874 (Table 1). No significant deviation from Hardy–Weinberg equilibrium after Bonferroni sequential correction for multiple comparisons was observed (Table 1). We successfully amplified 17 and 14 of our loci in *P. concolor* and *P. ponerophaga*, respectively (Table 2).

These microsatellites will be useful for assessing the role of introgression/hybridization in the success of a biological control introduction as undertaken within the framework of a new French research project. Well-designed

monitoring studies of field-released *P. lounsburyi* using these markers would undoubtedly benefit to ongoing biological control programmes of olive fruit flies in California in particular.

Acknowledgements

We are grateful to Kim Hoelmer, Alan Kirk, Arnaud Blanchet, Marie Roche, Nassera Kadiri and Beatrice Hurtrel for providing us with specimens of the studied species, and to Robert Wharton for taxonomic identification. This work was funded by the French Agence Nationale de la Recherche (ANR) biodiversity grant (BioInv-4I). We would like to thank the California Department of Food and Agriculture and US Department of Agriculture–Animal and Plant Health Inspection Service (USDA–APHIS) for their continuous financial support during these past years for exploration, collection and colonization of materials from Africa. Voucher specimens are deposited in EBCL core collection.

References

- Daane KM, Sime KR, Wang XG *et al.* (2008) *Psytalia lounsburyi* (Hymenoptera: Braconidae), potential biological control agent for the olive fruit fly in California. *Biological Control*, **44**, 79–89.
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, **19**, 11–15.
- Facon B, Genton BJ, Shykoff J, Jarne P, Estoup A, David P (2006) A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution*, **21**, 130–135.
- Fournier D, Foucaud J, Loiseau A *et al.* (2005) Characterization and PCR multiplexing of polymorphic microsatellite loci for the invasive ant *Wasmannia auropunctata*. *Molecular Ecology Notes*, **5**, 239–242.
- Hufbauer RA, Roderick GK (2005) Microevolution in biological control: mechanisms, patterns, and processes. *Biological Control*, **35**, 227–239.
- Kijas JMH, Fowler JCS, Garbet CA, Thomas MR (1994) Enrichment of microsatellites from citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *BioTechniques*, **16**, 657–662.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.